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Extending the functions of the homeotic transcription factor Cdx2 in the digestive system through nontranscriptional activities

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Abstract

The homeoprotein encoded by the intestinal-specific *Cdx2* gene is a major regulator of gut development and homeostasis, also involved in colon cancer as well as in intestinal-type metaplasias when it is abnormally expressed outside the gut. At the molecular level, structure/function studies have demonstrated that the Cdx2 protein is a transcription factor containing a conserved homeotic DNA-binding domain made of

three alpha helices arranged in a helix-turn-helix motif, preceded by a transcriptional domain and followed by a regulatory domain. The protein interacts with several thousand sites on the chromatin and widely regulates intestinal functions in stem/progenitor cells as well as in mature differentiated cells. Yet, this transcription factor also acts through original nontranscriptional mechanisms. Indeed, the identification of novel protein partners of Cdx2 and also of a splicing variant revealed unexpected functions in the control of signaling pathways like the Wnt and NF- κ B pathways, in double-strand break DNA repair and in premessenger RNA splicing. These novel functions of Cdx2 must be considered to fully understand the complexity of the role of Cdx2 in the healthy intestine and in diseases.

Key words: Homeobox; Transcription; Signaling; DNA repair; RNA splicing; Intestine; Development; Cancer; Homeostasis

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Core tip: The homeobox gene *Cdx2* plays crucial functions in the gut, being the one determinant of intestinal identity during embryonic development, and then a key regulator of homeostasis of the gut epithelium throughout adulthood. It acts at the level of the stem/progenitor cells as well as in mature epithelial cells. *Cdx2* is also important in digestive diseases, especially in colorectal cancer where it is thought to have a tumor suppressor role. In addition, it becomes abnormally expressed ectopically in non-intestinal organs, including gastric intestinal-type metaplasia and Barrett's esophagus and their related adenocarcinomas, and even in leukemia. The homeoprotein Cdx2 primarily acts as a DNA-binding transcription factor. However, recent reports provide evidence for novel mechanisms of action that are transcription-independent. In this

review, we summarize these new data that considerably extend the molecular potential of Cdx2 and open new questions and research area regarding the role of this homeoprotein in physiology and pathology.

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CDX2: A MAJOR DETERMINANT OF INTESTINAL DEVELOPMENT AND HOMEOSTASIS, ALSO CONTRIBUTING TO DIGESTIVE DISEASES

Homeobox genes, first identified in pioneer studies conducted in *Drosophila melanogaster*, encode evolutionary-conserved transcription factors that are key players of the embryonic development, especially for axis formation and patterning. Some of them remain expressed throughout adulthood to control tissue homeostasis, and therefore they might be involved in diseases. Although many homeobox genes are dispersed in the genome, others are clustered. Mammals have four *Hox* gene clusters, the clusters *HoxA* to *HoxD* gathering 9 to 11 genes, as well as one *paraHox* cluster made of 3 homeobox genes: *Gsx1*, *Pdx1* and *Cdx2* (*Caudal*-related homeobox); two additional *Cdx* genes are dispersed, *Cdx1* and *Cdx4* (see Ref 1 for a review of the organization and expression of homeobox genes). Here, we will focus on the *paraHox* homeobox gene *Cdx2*^[1].

The homeobox gene *Cdx2* exhibits a complex expression pattern described in detail in mice. It turns on in 8- to 32-cells zygotes to progressively concentrate in the trophectoderm^[2]. Embryonic tissues first express *Cdx2* after gastrulation around E8.5 at the level of the tail bud in the three germ layers: the ectoderm, the mesoderm and the endoderm of the hindgut rudiment. It extends anteriorly in a graded pattern in the neural tube, notochord and paraxial mesoderm, but shows a sharp boundary in the endoderm at the junction between the foregut and midgut. From midgestation onwards, *Cdx2* disappears in ectodermal and mesodermal derivatives, but remains selectively expressed throughout adult life in the midgut and hindgut endoderm forming the small intestine and colon epithelium^[3]. Distinct regions of the *Cdx2* promoter cooperate to generate this complex expression pattern^[4].

Functional studies have revealed the lethality of homozygous *Cdx2*^{-/-} knockout mutants as early as day 3.5 *pc*, which demonstrates the primordial role of this homeobox gene in the trophectoderm and its extra-embryonic derivatives^[5-7]. Then during early development of the embryo, *Cdx2* participates in the posterior elongation and patterning of the body^[8,9]. Further studies to determine the precise function(s) of *Cdx2* in the gut at later embryonic stages and postnatally were precluded by the very early lethality of *Cdx2*^{-/-} mutants, which therefore needed developing a conditional knockout approach. These studies have highlighted the key role played by this homeobox gene in the digestive tube. Indeed, the endoderm-specific ablation of *Cdx2* shortly after gastrulation or at midgestation results in embryonic/perinatal death associated to posterior gut truncations and to the transformation of the intestinal endoderm into esophageal-like or gastric-like epithelium; thus, *Cdx2* is a key actor of gut morphogenesis and the one gene that determines the tissue-identity of the presumptive intestinal endoderm^[10,11]. This conclusion drawn from animal models is corroborated by observations made in humans that show a close relationship between the presence or inversely the absence of *Cdx2* respectively in intestinal-type Meckel's diverticulum and in congenital gastric-type metaplasia of the gut^[12]. Beyond embryonic development, the continued expression of *Cdx2* is required throughout life in the continuously-renewing intestinal epithelium. Indeed, conditional inactivation in the adult gut epithelium leads to death, as a result of defects in cell renewal and in digestive functions, linked to a shift from intestinal-type to gastric-type differentiation^[13,14]. The function of *Cdx2* is relevant already in stem cells, which is underlined by the inability of knockout Crypt Base Columnar cells (CBC, Lgr5+ cells) to produce typical "mini-intestine" organoids *in vitro*, unlike wild type CBC cells^[14]. Conversely, *Cdx2* overexpression in the gut is also lethal because of an excessive epithelial maturation^[15]. Together, these results emphasize the complex role played by *Cdx2* in the adult gut epithelium: it provides the stem cells with the information needed for maintaining their intestinal identity, it participates in the cellular organization of the stem cell niche and in cell renewal, and it controls the terminal differentiation of the mature digestive cells.

In addition to its crucial role in intestinal homeostasis, *Cdx2* is important in pathologies, in particular in colon cancers. Its expression decreases and becomes heterogeneous in a significant proportion of tumors, which correlates with poor prognosis and reduced disease free survival^[16-18]. Experimentally in mice, a decline of *Cdx2* contributes to tumor progression^[19,20] and malignant cell dissemination^[21], leading to attribute a tumor sup-

pressor role to *Cdx2* in the gut. Besides, although *Cdx2* is physiologically restricted to the intestinal epithelium, its expression becomes ectopically turned on outside the gut in a number of epithelial organs in pathological settings associated with chronic inflammation. This is the case for instance of the gastroesophageal reflux in the esophagus and of *Helicobacter pylori* infection in the stomach^[22]. The ectopic expression correlates with the development of intestinal-type metaplasias, as observed in Barrett's esophagus and in gastric intestine-type metaplasia. In the latter case, transgenic mice have demonstrated that *Cdx2* is the actual driver of the abnormal intestinal transdifferentiation of the gastric mucosa^[23,24]. Intestinal-type metaplasia being generally considered as a precancerous lesion, this opens the question whether *Cdx2* could have a pro-oncogenic potential outside the gut. Yet, its expression tends to decrease during the pathological sequence from intestinal-type metaplasia to adenomas and carcinomas, and this decline is a factor of poor prognosis in gastric cancers^[25-28], suggesting a tumor suppressor activity after the onset of the cancerous process. Thus, whether *Cdx2* exerts a pro-oncogenic or a tumor suppressor activity, or both, in intestinal-type adenocarcinoma outside the gut is still under debate. Ectopic expression of *Cdx2* is also a frequent event in acute myeloid leukemia and acute lymphoblastic leukemia; in these cases, experimental evidences in mice support an oncogenic role^[29-30].

CDX2 HOMEOPROTEIN IS A TRANSCRIPTION FACTOR

Homeobox genes are characterized by a conserved 180-bp nucleotide sequence encoding the homeodomain of 60 amino-acids, rich in basic residues^[31]. This domain organizes into three alpha-helices arranged in a helix-turn-helix conformation, and constitutes the DNA-binding domain of the protein. In typical homeoproteins, this domain is preceded at the N-side by the transactivation domain and followed at the C-side by a domain of various lengths whose function is poorly documented; few homeoproteins present a second DNA-binding domain.

Following the initial discovery of a short element of the *Cdx2* gene by sequence homology search^[32], the full-length protein was characterized a few years later as a transcriptional activator of the insulin and sucrase-isomaltase gene promoters^[33,34]. Structure/function analyses have demonstrated its actual properties of DNA-binding transcription factor. The DNA interaction with the homeodomain was proven by Electromobility Shift and DNase protection assays^[34,35], the consensus *cis*-element being determined for Caudal-type homeoproteins

by SELEX [5'-(A/C)TTTAT(A/G)-3']^[36] and later refined for *Cdx2* by ChIP [5'-(A/C)N(A/T)N(T/A/G)(T/C)(T/A)A(T/C)(T/G/A)(G/A)(C/T)(C/A/T)-3']^[37]. The transcriptional activity of the domain located upstream of the homeodomain was established using chimeric and mutant proteins^[38,39]. This transactivation domain contains phosphorylation sites that positively or negatively regulate its activity, depending on the residues that is phosphorylated^[40,41]. In addition, the region of *Cdx2* located downstream of the homeodomain also contains a cluster of phosphorylation sites that constitutes a signal for ubiquitin- and proteasome-dependent degradation of the protein, and therefore that regulates its turnover^[42].

In addition to sucrase-isomaltase, the *Cdx2* homeoprotein regulates the promoter activity of a number of genes involved in various intestinal functions like digestive hydrolases and transporters (lactase, hephaestin, ASBT)^[43-45], mucins (Muc2, Muc4)^[46,47], cell adhesion molecules (Cadherin-17, Claudin-2, Mucdhl)^[48-50], receptors and signaling molecules^[51-53], regulatory peptides (proglucagon)^[54] and others. This gives *Cdx2* a central role in both absorptive and secretory lineages in the gut. More recently, a comprehensive study by ChIP-seq has revealed that *Cdx2* dynamically interacts with nearly 17000 chromatin binding sites across the whole genome, about 700 being specific of proliferating cells and about 14000 of differentiated cells^[37]. In both cellular states, *Cdx2* occupancy correlates with selective enhancer elements placed in an active chromatin conformation. Interestingly, those enhancers bound to *Cdx2* in differentiated cells are enriched in sites co-occupied by HNF4, whereas enhancers bound to *Cdx2* in proliferating cells are enriched in sites co-occupied by GATA6 and also by Tcf4, a crucial mediator of Wnt signaling required for stem cells maintenance in the gut^[37,55]. Thus, the co-occupancy of chromatin enhancers by *Cdx2* in combination with various transcription factors provides the rationale for understanding the transcriptional bases of the stepwise functions of this homeoprotein during the constant renewal of the intestinal epithelium, in stem cells, proliferating progenitors and mature differentiated cells. Altogether, these data establish at the molecular level the role of *Cdx2* as a DNA-binding transcription factor central for gut homeostasis.

NONTRANSCRIPTIONAL ACTIVITIES OF THE CDX2 HOMEOPROTEIN

Although the *Cdx2* protein is primarily a transcription factor, recent data have revealed novel mechanisms of action that are nontranscriptional. Compared to the DNA-binding-dependent transcriptional activity of *Cdx2*, we define below nontranscriptional activities

as activities being independent of DNA-binding or independent of transactivation.

Cell proliferation

In several human colon cancer cell lines, increasing the level of Cdx2 reduces cell proliferation^[21,56,57], which is at least in part due to the transcriptional activation of the promoter of the gene encoding the cyclin-dependent kinase inhibitor p21^{WAF158]}. Based on the observation that the hemizygous reduction of *Cdx2* in mice increases intestinal cell proliferation and also the susceptibility to colon cancer in *Apc*⁺⁷¹⁶ mutants^[20], Aoki *et al.*^[59] recently displayed a novel mechanism of cell cycling inhibition by Cdx2, independent of its transcriptional activity. Indeed, cell cycling inhibition is achieved as efficiently by the wild type protein Cdx2 and by mutants unable to bind the DNA. Moreover, mutants lacking the transactivation domain, although less efficient than the wild type protein, also reduce cell proliferation. The mechanism of inhibition is still not fully elucidated. However, evidence is provided that it results from the mRNA-independent increase of the level of cyclin-dependent kinase inhibitor p27^{Kip1} linked to its stabilization by blocking ubiquitination and degradation by the proteasome.

The Cyclin-dependent kinases and their inhibitors involved in the correct progression through the cell cycle are controlled by several signaling pathways that converge in stem cells and progenitors to fuel the constant renewal of the intestinal epithelium. The canonical Wnt pathway is a major player of this process that regulates downstream targets like the CyclinD1 and *c-myc* promoters *via* the formation of the bipartite transcriptional complex made of β -catenin bound to Tcf4. In addition to its role in intestinal homeostasis, this pathway is also a leading actor of colon tumorigenesis when over-activated. Genetic and functional interactions between the Wnt pathway and *Cdx2* are multiple and far from being fully elucidated. In this context, Guo *et al.*^[60] have reported that Cdx2 can interfere with this pathway in colon cancer cells to lessen cell proliferation. Mechanistically, the Cdx2 protein interacts with β -catenin, thus preventing the latter to recognize the DNA-binding factor Tcf4 and activate the pathway. The interaction between Cdx2 and β -catenin requires the region of the homeoprotein preceding the homeodomain but uses elements separate from those needed for transactivation; indeed, mutants in this domain deficient in transcriptional activity can still bind β -catenin and prevent its interaction with Tcf4. Therefore, these data illustrate a novel transcription-independent mechanism of action of Cdx2 whereby the homeoprotein interacts with and blocks the recruitment of a transcriptional activator on the chromatin.

A similar relationship has been described between

Cdx2 and another signaling pathway, NF- κ B, also relevant in the field of colon cancer. Beside the intrinsic oncogenic mutations affecting cell proliferation and apoptosis, the abnormal growth and invasive properties of tumor cells also result from altered interactions with their microenvironment. Prostaglandins, especially the E2 series (PGE2) synthesized from arachidonic acid by Cyclooxygenase-2 (Cox-2), accumulate in the microenvironment of the tumor cells and stimulate their proliferation and invasion potential. The source of PGE2 is multiple, including the malignant cells themselves. This is linked to the up-regulation of Cox-2 in tumor cells in response to several signaling pathways, among which the NF- κ B cascade. Interestingly, Kim and coworkers^[61], and other later^[62], have reported that Cdx2 inhibits Cox-2 expression and consequently the production of PGE2, in spite of the absence of *cis*-element for the binding of Cdx2 in the Cox-2 gene promoter. Instead, the Cdx2 homeoprotein achieves its effect by interacting with the p65 subunit of NF- κ B and preventing its binding to the corresponding DNA elements of the Cox-2 promoter. Thus, in the case of NF- κ B as shown above for the Wnt pathway, Cdx2 intercepts signaling mediators when they reach the nucleus to divert them from their chromatin targets and therefore interrupt the signaling cascade.

DNA repair

The *Cdx2* gene exerts a tumor suppressor activity at its physiological site of expression, the gut, but it expected to be pro-oncogenic when ectopically expressed in the hematopoietic lineage. In order to get an enlightenment of the molecular bases of these opposite functions, Renouf *et al.*^[63] have hypothesized that the ultimate output of Cdx2 could depend on the cellular context in a given tissue or in other words on specific partners interacting with the homeoprotein. Comparative proteomics identified the KU70/80 complex as a partner of Cdx2 selectively in colon cancer cells in contrast to leukemia cells, although KU70/80 is widely expressed in both cell types. KU70/80 is involved in a large panel of nuclear functions among which the recognition of double-strand DNA breaks (DSB) to initiate the complex molecular process leading to DNA repair. Functional studies have revealed that Cdx2, through its interaction with KU70/80, inhibits DSB DNA repair only in colon cancer cells but not in leukemia cells, and hence compromises colon cancer cell survival after a genotoxic stress. This inhibitory effect is associated to a reduction of the kinase activity of DNA-PKcs recruited into the repair complex by KU70/80 at the site of DNA break. Noteworthy, Cdx2 exerts its inhibitory effect independently of its transcriptional activity since a mutant devoid of transactivation domain is as efficient as the full-length homeoprotein. This non-

transcriptional function of Cdx2 on DSB DNA repair is particularly relevant from the perspective of its tumor suppressor role in the gut, especially with regard to the resistance to γ -irradiation-induced apoptosis^[19] and to the chromosomal instability^[20] observed when the Cdx2 level is decreased in the colon epithelium of *Cdx2*^{+/-} mice.

Pre-messenger RNA splicing

In a recent study conducted by Witek *et al.*^[64], a novel mRNA isoform expected to result from the alternative splicing of the *Cdx2* pre-messenger RNA has been described. Alternative splicing uses a non-conventional splicing donor site at the 5'-extremity of the second Intron which, in combination with the acceptor splicing site of this Intron, creates a 4-bases deletion compared to the classical *Cdx2* mRNA and hence a frame-shift. The resulting protein variant, Cdx2-AS, shares with Cdx2 the transactivation domain, the first two alpha-helices of the homeodomain but the 85-amino-acids region corresponding to the third alpha-helix and C-side domain of Cdx2 are replaced in Cdx2-AS by a 42-amino-acid domain unrelated in sequence to the C-end of Cdx2. The absence of the third alpha-helix, crucial for the interaction of the homeodomain with the major groove of DNA^[62], makes the Cdx2-AS variant a DNA-binding-inefficient protein. Consequently, this variant fails to transcriptionally activate typical promoter targets of Cdx2. Nonetheless, the new 42-amino-acids domain at the C-end of Cdx2-AS is rich in Serine and Arginine residues and presents some degree of sequence similarity with SR-proteins involved in RNA splicing. Functional studies established that it actually modulates the splicing pattern of genes expressed in gut cells. Therefore, these data point out the existence of an original splicing variant of *Cdx2* encoding a transcription-deficient nuclear factor that acts instead as a regulator of another major nuclear functions: the processing of pre-messenger RNAs.

OPEN QUESTIONS

The data summarized above strongly support that Cdx2 exerts transcription-independent functions, as already shown for other transcription factors as important as p53, c-Myc and E2F1^[65-67], as well as for few homeoproteins of the Hox family^[68]. Most of the examples illustrating here the transcription-independent functions of Cdx2 are related to clinical situations, especially cancer. In particular, the inhibitory effect of Cdx2 on Wnt signaling has been studied in the context of a constitutively activated (oncogenic) form of β -catenin^[60], while the effect on DSB DNA repair was evaluated in the perspective of the treatment of cancer cells with potential genotoxic drugs. It is thus important, for the future, to address whether the balance between

the transcriptional and nontranscriptional functions of Cdx2 is stable or if it changes in clinical situations (colon cancer, inflammatory bowel diseases, metaplasia related to the ectopic expression of *Cdx2*, leukemia) compared to the physiological situation, or in other words if the nontranscriptional functions of Cdx2 become particularly relevant in diseases and/or in response to therapeutic treatments. In addition, it would also be interesting to decipher and compare the respective roles of the transcription-independent vs -dependent functions of Cdx2 in the sequential cellular states characterizing the constant renewal of the intestinal epithelium, in stem cells, committed progenitors and postmitotic differentiated cells. These issues can also be raised for the developmental roles of *Cdx2*, in the presumptive gut, in body axis elongation and patterning, and even in extraembryonic tissues.

The nontranscriptional functions of Cdx2 are largely related to the capacity of the homeoprotein to interact with a variety of partners. Proteomics technology has substantially improved during the last years allowing investigating protein-protein interactions at a large scale; however no comprehensive study has been performed so far to characterize the interactome of Cdx2. This objective is challenging because such studies can be complicated by the fact that molecular interactions are strongly reliant on the cellular context. The context is defined on the one hand by the panel of putative Cdx2 partners expressed within a given cell, and on the other hand by the posttranslational modifications of Cdx2 and its partners that condition the physical interactions. Therefore, characterizing the transcription-independent activities of Cdx2 not only requires identifying its multiple interacting proteins but also determining the pattern of posttranslational modifications. The Cdx2 protein has already been shown to be ubiquitinated and phosphorylated^[40-42], the phosphorylation at different sites producing different effects. Additional types of modifications are also possible like acetylation, methylation, PARYlation and others. Conversely, based on similar considerations, another stimulating issue is to know if Cdx2, when it interacts with a given partner, modulates all the functions attributed to this partner or only a subset of these functions. For instance, the KU70/80 complex has many roles beyond DSB DNA repair, including the regulation of gene expression, the preservation of telomeres and the control of apoptosis. It is not known yet if Cdx2, through KU70/80, may also belong to and affect the molecular complexes containing proteins involved in these functions, like hTERT and Bax.

Molecular interactions between partners are highly dependent on the compartmentalization of proteins. This issue is particularly relevant to connect post-translational modifications with diseases. Interestingly, whereas the Cdx2 protein is restricted to the nucleus in normal intestinal cells, a diffuse

cytoplasmic immunostaining often appears in histological sections of human colorectal tumors and already in a number of adenomas, suggesting that a fraction of the homeoprotein is abnormally redirected outside its physiological compartment. This could be related to an unusual form of posttranslational modification and was generally considered as a sign of inactivity because the transcription factor is remote from the chromatin. However, since Cdx2 also has transcription-independent functions, one can now speculate that the homeoprotein meets new partners in the odd location and therefore affects a subset of cytoplasmic functions specifically in disease cells, that cannot be anticipated from studies conducted in healthy cells. Investigating this issue is important but challenging because, to our knowledge, no human colon cancer cell line has been reported so far to recapitulate the diffuse distribution of Cdx2 in the cytoplasm; without appropriate model, this makes it difficult to mechanistically approach the role of cytoplasmic Cdx2 in malignant cells.

CONCLUSION

In conclusion, beside its primarily role of transcription factor, more and more data support the emerging concept that the Cdx2 homeoprotein also exerts transcription-independent activities. These activities considerably broaden the field and the mechanisms of action of this factor, being now involved in new and important cellular and molecular functions, like DNA repair and RNA splicing. These functions were not previously anticipated from the transcriptomic data related to Cdx2. Determining the complete list of functions regulated by Cdx2 using a nontranscriptional mechanism, together with the list of Cdx2 partners for these functions, is a very ambitious objective at the molecular and cellular levels, and it is even more challenging to put these nontranscriptional functions into a physiological or pathological context with appropriate genetic models in mice. These objectives stimulate new directions of research, the results of which should extend our knowledge on the development and homeostasis of the gut as well as on malignant and/or inflammatory diseases.

REFERENCES

- 1 **Garcia-Fernández J**. The genesis and evolution of homeobox gene clusters. *Nat Rev Genet* 2005; **6**: 881-892 [PMID: 16341069 DOI: 10.1038/nrg1723]
- 2 **Beck F**, Erler T, Russell A, James R. Expression of Cdx-2 in the mouse embryo and placenta: possible role in patterning of the extra-embryonic membranes. *Dev Dyn* 1995; **204**: 219-227 [PMID: 8573715 DOI: 10.1002/aja.1002040302]
- 3 **Silberg DG**, Swain GP, Suh ER, Traber PG. Cdx1 and cdx2 expression during intestinal development. *Gastroenterology* 2000; **119**: 961-971 [PMID: 11040183 DOI: 10.1053/gast.2000.18142]
- 4 **Benahmed F**, Gross I, Gaunt SJ, Beck F, Jehan F, Domon-Dell C, Martin E, Kedinger M, Freund JN, Duluc I. Multiple regulatory regions control the complex expression pattern of the mouse Cdx2 homeobox gene. *Gastroenterology* 2008; **135**: 1238-1247, 1247.e1-3 [PMID: 18655789 DOI: 10.1053/j.gastro.2008.06.045]
- 5 **Chawengsaksophak K**, James R, Hammond VE, Köntgen F, Beck F. Homeosis and intestinal tumours in Cdx2 mutant mice. *Nature* 1997; **386**: 84-87 [PMID: 9052785 DOI: 10.1038/386084a0]
- 6 **Niwa H**, Toyooka Y, Shimosato D, Strumpf D, Takahashi K, Yagi R, Rossant J. Interaction between Oct3/4 and Cdx2 determines trophectoderm differentiation. *Cell* 2005; **123**: 917-929 [PMID: 16325584 DOI: 10.1016/j.cell.2005.08.040]
- 7 **Ralston A**, Rossant J. Cdx2 acts downstream of cell polarization to cell-autonomously promote trophectoderm fate in the early mouse embryo. *Dev Biol* 2008; **313**: 614-629 [PMID: 18067887 DOI: 10.1016/j.ydbio.2007.10.054]
- 8 **van den Akker E**, Forlani S, Chawengsaksophak K, de Graaff W, Beck F, Meyer BI, Deschamps J. Cdx1 and Cdx2 have overlapping functions in anteroposterior patterning and posterior axis elongation. *Development* 2002; **129**: 2181-2193 [PMID: 11959827]
- 9 **Chawengsaksophak K**, de Graaff W, Rossant J, Deschamps J, Beck F. Cdx2 is essential for axial elongation in mouse development. *Proc Natl Acad Sci USA* 2004; **101**: 7641-7645 [PMID: 15136723 DOI: 10.1073/pnas.0401654101]
- 10 **Gao N**, White P, Kaestner KH. Establishment of intestinal identity and epithelial-mesenchymal signaling by Cdx2. *Dev Cell* 2009; **16**: 588-599 [PMID: 19386267 DOI: 10.1016/j.devcel.2009.02.010]
- 11 **Grainger S**, Savory JG, Lohnes D. Cdx2 regulates patterning of the intestinal epithelium. *Dev Biol* 2010; **339**: 155-165 [PMID: 20043902 DOI: 10.1016/j.ydbio.2009.12.025]
- 12 **Martin E**, Vanier M, Taviani M, Guerin JN, Domon-Dell C, Duluc I, Gross I, Rowland J, Kim S, Freund JN. CDX2 in congenital gut gastric-type heteroplasia and intestinal-type Meckel diverticula. *Pediatrics* 2010; **126**: e723-e727 [PMID: 20679295 DOI: 10.1542/peds.2009-3512]
- 13 **Verzi MP**, Shin H, Ho LL, Liu XS, Shivdasani RA. Essential and redundant functions of caudal family proteins in activating adult intestinal genes. *Mol Cell Biol* 2011; **31**: 2026-2039 [PMID: 21402776 DOI: 10.1128/MCB.01250-10]
- 14 **Stringer EJ**, Duluc I, Saandi T, Davidson I, Bialecka M, Sato T, Barker N, Clevers H, Pritchard CA, Winton DJ, Wright NA, Freund JN, Deschamps J, Beck F. Cdx2 determines the fate of postnatal intestinal endoderm. *Development* 2012; **139**: 465-474 [PMID: 22190642 DOI: 10.1242/dev.070722]
- 15 **Crissey MA**, Guo RJ, Funakoshi S, Kong J, Liu J, Lynch JP. Cdx2 levels modulate intestinal epithelium maturity and Paneth cell development. *Gastroenterology* 2011; **140**: 517-528.e8 [PMID: 21081128 DOI: 10.1053/j.gastro.2010.11.033]
- 16 **Brabletz T**, Spaderna S, Kolb J, Hlubek F, Faller G, Bruns CJ, Jung A, Nentwich J, Duluc I, Domon-Dell C, Kirchner T, Freund JN. Down-regulation of the homeodomain factor Cdx2 in colorectal cancer by collagen type I: an active role for the tumor environment in malignant tumor progression. *Cancer Res* 2004; **64**: 6973-6977 [PMID: 15466189 DOI: 10.1158/0008-5472.CAN-04-1132]
- 17 **Baba Y**, Noshio K, Shima K, Freed E, Irahara N, Philips J, Meyerhardt JA, Hornick JL, Shivdasani RA, Fuchs CS, Ogino S. Relationship of CDX2 loss with molecular features and prognosis in colorectal cancer. *Clin Cancer Res* 2009; **15**: 4665-4673 [PMID: 19584150 DOI: 10.1158/1078-0432.CCR-09-0401]
- 18 **De Sousa E Melo F**, Wang X, Jansen M, Fessler E, Trinh A, de Rooij LP, de Jong JH, de Boer OJ, van Leersum R, Bijlsma MF, Rodermond H, van der Heijden M, van Noesel CJ, Tuynman JB, Dekker E, Markowitz F, Medema JP, Vermeulen L. Poor-prognosis colon cancer is defined by a molecularly distinct subtype and develops from serrated precursor lesions. *Nat Med* 2013; **19**: 614-618 [PMID: 23584090 DOI: 10.1038/nm.3174]
- 19 **Bonhomme C**, Duluc I, Martin E, Chawengsaksophak K, Chenard MP, Kedinger M, Beck F, Freund JN, Domon-Dell C. The Cdx2 homeobox gene has a tumour suppressor function in the distal colon in addition to a homeotic role during gut development.

- Gut* 2003; **52**: 1465-1471 [PMID: 12970140 DOI: 10.1136/gut.52.10.1465]
- 20 **Aoki K**, Tamai Y, Horiike S, Oshima M, Taketo MM. Colonic polyposis caused by mTOR-mediated chromosomal instability in Apc^{+/Delta716} Cdx2^{+/-} compound mutant mice. *Nat Genet* 2003; **35**: 323-330 [PMID: 14625550 DOI: 10.1038/ng1265]
 - 21 **Gross I**, Duluc I, Benameur T, Calon A, Martin E, Brabletz T, Kedinger M, Domon-Dell C, Freund JN. The intestine-specific homeobox gene Cdx2 decreases mobility and antagonizes dissemination of colon cancer cells. *Oncogene* 2008; **27**: 107-115 [PMID: 17599044 DOI: 10.1038/sj.onc.1210601]
 - 22 **Moskaluk CA**, Zhang H, Powell SM, Cerilli LA, Hampton GM, Frierson HF. Cdx2 protein expression in normal and malignant human tissues: an immunohistochemical survey using tissue microarrays. *Mod Pathol* 2003; **16**: 913-919 [PMID: 13679455 DOI: 10.1097/01.MP.0000086073.92773.55]
 - 23 **Silberg DG**, Sullivan J, Kang E, Swain GP, Moffett J, Sund NJ, Sackett SD, Kaestner KH. Cdx2 ectopic expression induces gastric intestinal metaplasia in transgenic mice. *Gastroenterology* 2002; **122**: 689-696 [PMID: 11875002 DOI: 10.1053/gast.2002.31902]
 - 24 **Mutoh H**, Hakamata Y, Sato K, Eda A, Yanaka I, Honda S, Osawa H, Kaneko Y, Sugano K. Conversion of gastric mucosa to intestinal metaplasia in Cdx2-expressing transgenic mice. *Biochem Biophys Res Commun* 2002; **294**: 470-479 [PMID: 12051735]
 - 25 **Park do Y**, Srivastava A, Kim GH, Mino-Kenudson M, Deshpande V, Zukerberg LR, Song GA, Lauwers GY. CDX2 expression in the intestinal-type gastric epithelial neoplasia: frequency and significance. *Mod Pathol* 2010; **23**: 54-61 [PMID: 19820687 DOI: 10.1038/modpathol.2009.135]
 - 26 **Seno H**, Oshima M, Taniguchi MA, Usami K, Ishikawa TO, Chiba T, Taketo MM. CDX2 expression in the stomach with intestinal metaplasia and intestinal-type cancer: Prognostic implications. *Int J Oncol* 2002; **21**: 769-774 [PMID: 12239615 DOI: 10.3892/ijo.21.4.769]
 - 27 **Xin S**, Huixin C, Benchang S, Aiping B, Jinhui W, Xiaoyan L, Yu WB, Minhu C. Expression of Cdx2 and claudin-2 in the multistage tissue of gastric carcinogenesis. *Oncology* 2007; **73**: 357-365 [PMID: 18500171 DOI: 10.1159/000135351]
 - 28 **Zhang X**, Tsukamoto T, Mizoshita T, Ban H, Suzuki H, Toyoda T, Tatematsu M. Expression of osteopontin and CDX2: indications of phenotypes and prognosis in advanced gastric cancer. *Oncol Rep* 2009; **21**: 609-613 [PMID: 19212618]
 - 29 **Scholl C**, Bansal D, Döhner K, Eiwien K, Huntly BJ, Lee BH, Rücker FG, Schlenk RF, Bullinger L, Döhner H, Gilliland DG, Fröhling S. The homeobox gene CDX2 is aberrantly expressed in most cases of acute myeloid leukemia and promotes leukemogenesis. *J Clin Invest* 2007; **117**: 1037-1048 [PMID: 17347684 DOI: 10.1172/JCI30182]
 - 30 **Thoene S**, Rawat VP, Heilmeyer B, Hoster E, Metzeler KH, Herold T, Hiddemann W, Gökbuget N, Hoelzer D, Bohlander SK, Feuring-Buske M, Buske C. The homeobox gene CDX2 is aberrantly expressed and associated with an inferior prognosis in patients with acute lymphoblastic leukemia. *Leukemia* 2009; **23**: 649-655 [PMID: 19158837 DOI: 10.1038/leu.2008.355]
 - 31 **Moreland RT**, Ryan JF, Pan C, Baxevanis AD. The Homeo-domain Resource: a comprehensive collection of sequence, structure, interaction, genomic and functional information on the homeodomain protein family. *Database* (Oxford) 2009; **2009**: bap004 [PMID: 20157477 DOI: 10.1093/database/bap004]
 - 32 **James R**, Kazenwadel J. Homeobox gene expression in the intestinal epithelium of adult mice. *J Biol Chem* 1991; **266**: 3246-3251 [PMID: 1671571]
 - 33 **German MS**, Wang J, Chadwick RB, Rutter WJ. Synergistic activation of the insulin gene by a LIM-homeo domain protein and a basic helix-loop-helix protein: building a functional insulin minienhancer complex. *Genes Dev* 1992; **6**: 2165-2176 [PMID: 1358758 DOI: 10.1101/gad.6.11.2165]
 - 34 **Suh E**, Chen L, Taylor J, Traber PG. A homeodomain protein related to caudal regulates intestine-specific gene transcription. *Mol Cell Biol* 1994; **14**: 7340-7351 [PMID: 7935448 DOI: 10.1128/
 - MCB.14.11.7340]
 - 35 **Traber PG**, Wu GD, Wang W. Novel DNA-binding proteins regulate intestine-specific transcription of the sucrase-isomaltase gene. *Mol Cell Biol* 1992; **12**: 3614-3627 [PMID: 1378530 DOI: 10.1128/MCB.12.8.3614]
 - 36 **Margalit Y**, Yarus S, Shapira E, Gruenbaum Y, Fainsod A. Isolation and characterization of target sequences of the chicken CdxA homeobox gene. *Nucleic Acids Res* 1993; **21**: 4915-4922 [PMID: 7909943]
 - 37 **Verzi MP**, Shin H, He HH, Sulahian R, Meyer CA, Montgomery RK, Fleet JC, Brown M, Liu XS, Shivdasani RA. Differentiation-specific histone modifications reveal dynamic chromatin interactions and partners for the intestinal transcription factor CDX2. *Dev Cell* 2010; **19**: 713-726 [PMID: 21074721 DOI: 10.1016/j.devcel.2010.10.006]
 - 38 **Taylor JK**, Levy T, Suh ER, Traber PG. Activation of enhancer elements by the homeobox gene Cdx2 is cell line specific. *Nucleic Acids Res* 1997; **25**: 2293-2300 [PMID: 9171078]
 - 39 **Trinh KY**, Jin T, Drucker DJ. Identification of domains mediating transcriptional activation and cytoplasmic export in the caudal homeobox protein Cdx-3. *J Biol Chem* 1999; **274**: 6011-6019 [PMID: 10026228 DOI: 10.1074/jbc.274.9.6011]
 - 40 **Rings EH**, Boudreau F, Taylor JK, Moffett J, Suh ER, Traber PG. Phosphorylation of the serine 60 residue within the Cdx2 activation domain mediates its transactivation capacity. *Gastroenterology* 2001; **121**: 1437-1450 [PMID: 11729123 DOI: 10.1053/gast.2001.29618]
 - 41 **Houde M**, Laprise P, Jean D, Blais M, Asselin C, Rivard N. Intestinal epithelial cell differentiation involves activation of p38 mitogen-activated protein kinase that regulates the homeobox transcription factor CDX2. *J Biol Chem* 2001; **276**: 21885-21894 [PMID: 11283019 DOI: 10.1074/jbc.M100236200]
 - 42 **Gross I**, Lhermitte B, Domon-Dell C, Duluc I, Martin E, Gaididon C, Kedinger M, Freund JN. Phosphorylation of the homeotic tumor suppressor Cdx2 mediates its ubiquitin-dependent proteasome degradation. *Oncogene* 2005; **24**: 7955-7963 [PMID: 16027724 DOI: 10.1038/sj.onc.1208945]
 - 43 **Troelsen JT**, Mitchelmore C, Spodsberg N, Jensen AM, Norén O, Sjöström H. Regulation of lactase-phlorizin hydrolase gene expression by the caudal-related homeodomain protein Cdx-2. *Biochem J* 1997; **322** (Pt 3): 833-838 [PMID: 9148757]
 - 44 **Hinoi T**, Gesina G, Akyol A, Kuick R, Hanash S, Giordano TJ, Gruber SB, Fearon ER. CDX2-regulated expression of iron transport protein hephaestin in intestinal and colonic epithelium. *Gastroenterology* 2005; **128**: 946-961 [PMID: 15825077 DOI: 10.1053/j.gastro.2005.01.003]
 - 45 **Ma L**, Jüttner M, Kullak-Ublick GA, Eloranta JJ. Regulation of the gene encoding the intestinal bile acid transporter ASBT by the caudal-type homeobox proteins CDX1 and CDX2. *Am J Physiol Gastrointest Liver Physiol* 2012; **302**: G123-G133 [PMID: 22016432 DOI: 10.1152/ajpgi.00102.2011]
 - 46 **Mesquita P**, Jonckheere N, Almeida R, Ducourouble MP, Serpa J, Silva E, Pigny P, Silva FS, Reis C, Silberg D, Van Seuningen I, David L. Human MUC2 mucin gene is transcriptionally regulated by Cdx homeodomain proteins in gastrointestinal carcinoma cell lines. *J Biol Chem* 2003; **278**: 51549-51556 [PMID: 14525978 DOI: 10.1074/jbc.M309019200]
 - 47 **Jonckheere N**, Vincent A, Perrais M, Ducourouble MP, Male AK, Aubert JP, Pigny P, Carraway KL, Freund JN, Renes IB, Van Seuningen I. The human mucin MUC4 is transcriptionally regulated by caudal-related homeobox, hepatocyte nuclear factors, forkhead box A, and GATA endodermal transcription factors in epithelial cancer cells. *J Biol Chem* 2007; **282**: 22638-22650 [PMID: 17553805 DOI: 10.1074/jbc.M700905200]
 - 48 **Hinoi T**, Lucas PC, Kuick R, Hanash S, Cho KR, Fearon ER. CDX2 regulates liver intestine-cadherin expression in normal and malignant colon epithelium and intestinal metaplasia. *Gastroenterology* 2002; **123**: 1565-1577 [PMID: 12404231 DOI: 10.1053/gast.2002.36598]
 - 49 **Sakaguchi T**, Gu X, Golden HM, Suh E, Rhoads DB, Reinecker

- HC. Cloning of the human claudin-2 5'-flanking region revealed a TATA-less promoter with conserved binding sites in mouse and human for caudal-related homeodomain proteins and hepatocyte nuclear factor-1alpha. *J Biol Chem* 2002; **277**: 21361-21370 [PMID: 11934881 DOI: 10.1074/jbc.M110261200]
- 50 **Hinkel I**, Duluc I, Martin E, Guenot D, Freund JN, Gross I. Cdx2 controls expression of the protocadherin Mucdhl, an inhibitor of growth and β -catenin activity in colon cancer cells. *Gastroenterology* 2012; **142**: 875-885.e3 [PMID: 22202456 DOI: 10.1053/j.gastro.2011.12.037]
- 51 **Uesaka T**, Kageyama N, Watanabe H. Identifying target genes regulated downstream of Cdx2 by microarray analysis. *J Mol Biol* 2004; **337**: 647-660 [PMID: 15019784 DOI: 10.1016/j.jmb.2004.01.061]
- 52 **Modica S**, Morgano A, Salvatore L, Petruzzelli M, Vanier MT, Valanzano R, Esposito DL, Palasciano G, Duluc I, Freund JN, Mariani-Costantini R, Moschetta A. Expression and localisation of insulin receptor substrate 2 in normal intestine and colorectal tumours. Regulation by intestine-specific transcription factor CDX2. *Gut* 2009; **58**: 1250-1259 [PMID: 19221108 DOI: 10.1136/gut.2008.158386]
- 53 **Modica S**, Cariello M, Morgano A, Gross I, Vegliante MC, Murzilli S, Salvatore L, Freund JN, Sabbà C, Moschetta A. Transcriptional regulation of the intestinal nuclear bile acid farnesoid X receptor (FXR) by the caudal-related homeobox 2 (CDX2). *J Biol Chem* 2014; **289**: 28421-28432 [PMID: 25138215]
- 54 **Jin T**, Drucker DJ. Activation of proglucagon gene transcription through a novel promoter element by the caudal-related homeodomain protein cdx-2/3. *Mol Cell Biol* 1996; **16**: 19-28 [PMID: 8524295]
- 55 **Verzi MP**, Hatzis P, Sulahian R, Philips J, Schuijers J, Shin H, Freed E, Lynch JP, Dang DT, Brown M, Clevers H, Liu XS, Shivdasani RA. TCF4 and CDX2, major transcription factors for intestinal function, converge on the same cis-regulatory regions. *Proc Natl Acad Sci USA* 2010; **107**: 15157-15162 [PMID: 20696899 DOI: 10.1073/pnas.1003822107]
- 56 **Lorentz O**, Duluc I, Arcangelis AD, Simon-Assmann P, Kedinger M, Freund JN. Key role of the Cdx2 homeobox gene in extracellular matrix-mediated intestinal cell differentiation. *J Cell Biol* 1997; **139**: 1553-1565 [PMID: 9396760 DOI: 10.1083/jcb.139.6.1553]
- 57 **Mallo GV**, Soubeyran P, Lissitzky JC, André F, Farnarier C, Marvaldi J, Dagorn JC, Iovanna JL. Expression of the Cdx1 and Cdx2 homeotic genes leads to reduced malignancy in colon cancer-derived cells. *J Biol Chem* 1998; **273**: 14030-14036 [PMID: 9593754 DOI: 10.1074/jbc.273.22.14030]
- 58 **Bai YQ**, Miyake S, Iwai T, Yuasa Y. CDX2, a homeobox transcription factor, upregulates transcription of the p21/WAF1/CIP1 gene. *Oncogene* 2003; **22**: 7942-7949 [PMID: 12970742 DOI: 10.1038/sj.onc.1206634]
- 59 **Aoki K**, Kakizaki F, Sakashita H, Manabe T, Aoki M, Taketo MM. Suppression of colonic polyposis by homeoprotein CDX2 through its nontranscriptional function that stabilizes p27Kip1. *Cancer Res* 2011; **71**: 593-602 [PMID: 21224344 DOI: 10.1158/0008-5472.CAN-10-2842]
- 60 **Guo RJ**, Funakoshi S, Lee HH, Kong J, Lynch JP. The intestine-specific transcription factor Cdx2 inhibits beta-catenin/TCF transcriptional activity by disrupting the beta-catenin-TCF protein complex. *Carcinogenesis* 2010; **31**: 159-166 [PMID: 19734199 DOI: 10.1093/carcin/bgp213]
- 61 **Kim SP**, Park JW, Lee SH, Lim JH, Jang BC, Lee SH, Jang IH, Freund JN, Suh SI, Mun KC, Song DK, Ha EM, Lee WJ, Kwon TK. Homeodomain protein CDX2 regulates COX-2 expression in colorectal cancer. *Biochem Biophys Res Commun* 2004; **315**: 93-99 [PMID: 15013430 DOI: 10.1016/j.bbrc.2004.01.020]
- 62 **Mutoh H**, Hayakawa H, Sakamoto H, Sugano K. Homeobox protein CDX2 reduces Cox-2 transcription by inactivating the DNA-binding capacity of nuclear factor-kappaB. *J Gastroenterol* 2007; **42**: 719-729 [PMID: 17876541 DOI: 10.1007/s00535-007-2088-y]
- 63 **Renouf B**, Soret C, Saandi T, Delalande F, Martin E, Vanier M, Duluc I, Gross I, Freund JN, Domon-Dell C. Cdx2 homeoprotein inhibits non-homologous end joining in colon cancer but not in leukemia cells. *Nucleic Acids Res* 2012; **40**: 3456-3469 [PMID: 22189105 DOI: 10.1093/nar/gkr1242]
- 64 **Witek ME**, Snook AE, Lin JE, Blomain ES, Xiang B, Magee M, Waldman SA. A novel CDX2 isoform regulates alternative splicing. *PLoS One* 2014; **9**: e104293 [PMID: 25101906 DOI: 10.1371/journal.pone.0104293]
- 65 **Speidel D**. Transcription-independent p53 apoptosis: an alternative route to death. *Trends Cell Biol* 2010; **20**: 14-24 [PMID: 19879762 DOI: 10.1016/j.tcb.2009.10.002]
- 66 **Cole MD**, Cowling VH. Transcription-independent functions of MYC: regulation of translation and DNA replication. *Nat Rev Mol Cell Biol* 2008; **9**: 810-815 [PMID: 18698328 DOI: 10.1038/nrm2467]
- 67 **Biswas AK**, Johnson DG. Transcriptional and nontranscriptional functions of E2F1 in response to DNA damage. *Cancer Res* 2012; **72**: 13-17 [PMID: 22180494 DOI: 10.1158/0008-5472.CAN-11-2196]
- 68 **Kusser W**, Zimmer K, Fiedler F. Characteristics of the binding of aminoglycoside antibiotics to teichoic acids. A potential model system for interaction of aminoglycosides with polyanions. *Eur J Biochem* 1985; **151**: 601-605 [PMID: 2411558 DOI: 10.1002/dvdy.24060]

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